Etiology of Parsley Damping-off and Influence of Temperature on Disease Development

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ABSTRACT

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Damping-off of seedlings resulted in extensive stand reductions of parsley in southern New Jersey during the 1981–1982 growing seasons. The fungi most frequently isolated from field-grown seedlings with symptoms of postemergence damping-off were *Fusarium oxysporum, F. solani, Pythium irregulare, P. ultimum,* and *Rhizoctonia solani* (AG-4, AG-1, and AG-2, type 2). In pathogenicity tests, most isolates of *P. irregulare* and *P. ultimum* were highly pathogenic and caused extensive preemergence and postemergence damping-off. Similarly, most isolates of *R. solani* AG-4 and AG-2, type 2, were highly pathogenic. Isolates of AG-2, however, varied considerably in pathogenicity. No other fungi tested for pathogenicity incited disease in parsley. In sand bed studies, a combination of *P. irregulare* and *P. ultimum* isolates was highly pathogenic at 15, 23, and 30 C. *R. solani*, however, was much less pathogenic at 15 C than at either 23 or 30 C. A similar response was obtained in experiments in environmental chambers.

Parsley (*Petroselinum crispum* (Mill.) Nym.) is an important crop in southern New Jersey. It is typically grown from March through September, although it is frequently overwintered to provide for the early spring market. Plain- and curl-leaved cultivars are grown in New Jersey and are marketed primarily as bunches for seasoning and garnishing foods.

One of the primary factors limiting parsley production is damping-off of seedlings. Damping-off results in poor stand densities, which prompts growers to seed fields at elevated rates as a means of moderating the impact of disease.

Although few reports exist concerning the etiology of parsley damping-off, *Alternaria* spp. (4), *Fusarium* spp. (23), *Pythium* spp. (2,16), *Rhizoctonia solani* Kühn (24), and *Sclerotinia sclerotiorum* (Lib.) de Bary (14) have been implicated as pathogens. In New Jersey, however, documentation of the prevalence, etiology, and epidemiology of this disease is lacking.

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The objectives of this study were to sample the major parsley-growing regions of southern New Jersey for postemergence damping-off of seedlings, determine the etiology of the disease, and investigate the relationship between temperature and disease development in controlled environments.

MATERIALS AND METHODS

Field sampling. Over a 7-mo period from March through September 1981, 87 parsley fields on 10 farms in Atlantic and Cumberland counties, New Jersey, were sampled for seedlings showing symptoms of postemergence damping-off. Where possible, 60 seedlings showing typical disease symptoms were randomly selected from each field for use in isolating pathogens. Collected plants were washed in running tap water for 0.5–2 hr to free roots of adhering soil. All plants were then stored in plastic bags at 4 C for a maximum of 1 wk before further use.

Isolation of fungi. To isolate fungi, tissue sections 1 cm or shorter were excised from the borders of advancing lesions and either washed in three successive baths of sterile distilled water or surface-sterilized for 10–30 sec in a 10% solution of 5.25% sodium hypochlorite (Clorox). Both surface-sterilized and water-rinsed tissue sections were blotted dry on autoclaved paper towels and plated (five sections per plate) on 2% water agar (WA) under laboratory conditions (22–24 C) for 24–72 hr. Fungi that grew from tissue sections were transferred to acidified potato-dextrose agar (APDA) (4 ml, 25% lactic acid per liter of medium) and identified. Isolates of *Pythium* spp. were speciated using the WA/sweet-corn method described by Lumsden et al (12) and the taxonomic criteria of Middleton (15). The key and methods described by Toussoun and Nelson (22) were used to identify *Fusarium* species.

***Rhizoctonia* anastomosis grouping.** Thirty-two randomly selected isolates of *R. solani* were assigned to anastomosis groupings (AG), using the precedure described by Burpee et al (1). Each isolate was paired with two isolates of each anastomosis group (obtained from D. K. Bell, University of Georgia). Two testers of each group were used because it is known that some isolates may anastomose with one tester and not with another within the same group (D. K. Bell, *personal communication*). All isolates were maintained on the PDYCA medium (39 g of Difco dehydrated PDA, 0.5 g of yeast extract, 0.5 g of casein hydrolysate, 2 g of agar, and 958 ml of deionized water) developed by Sumner and Bell (20).

Pathogenicity of isolates. Before pathogenicity testing, identified fungi were grouped according to their morphological characteristics on APDA. Several morphologically similar isolates from each genus or species were then selected at random for use in determining their relative pathogenicity on parsley.

Each isolate was propagated on 3% cornmeal-sand (w/w, CMS) for 7–14 days. Pasteurized field soil (74 C, 24 hr) was then infested with CMS-fungal cultures at a rate of 1:100 (w/w, CMS inoculum/soil). Controls were pasteurized soil mixed with sterile CMS.

Infested soil was placed in 10-cm paper pots and seeded with the curl-leaved parsley cultivar Forest Green and the plain-leaved cultivar Plain Italian at rates of 0.31 g/10-cm row. Each treatment was replicated four times. Pots were maintained in the greenhouse (25–30 C) and rated for disease 2 wk after seedling emergence, then 10 plants from each treatment were randomly selected for use in reisolating test fungi.

Temperature-pathogenicity studies. The relationship between temperature and the pathogenicity of *R. solani* and a combination of *Pythium irregulare* Buis. and *P. ultimum* Trow. (*P. irregulare-P. ultimum*) was determined in temperature-controlled sand beds and environmental chambers.

In sand bed studies, three wooden frames (57.5 cm wide × 152.5 cm long × 12.5 cm high) were fitted with rubber

propagation mats (55 × 150 cm) (E. C. Geiger, Harleysville, PA) and filled to a depth of about 10 cm with a mixture of one part vermiculite and five parts washed sand. The temperatures of beds were controlled using 1,800W/15-amp coil thermostats, accurate within 1 C (E. C. Geiger, Harleysville, PA). The sensor bulb of each thermostat was buried in the top 2 cm of a soil-filled pot sunk into the center of each of the three beds.

Individual sand beds were maintained at 15, 23, and 30 C, respectively, and held in a greenhouse at 10–15 C, thereby allowing the low-temperature bed to stabilize at 15 C. The high-temperature bed was covered with bedding plastic to permit a temperature of 30 C to be reached despite the relatively low ambient temperature of the greenhouse. All beds were soaked with water and allowed to stabilize for 1 wk before further use.

Paper pots 10 cm in diameter were then infested with cornmeal-sand cultures of either *R. solani* AG-4 (1.5% w/w, CMS/soil) or *P. irregulare-P. ultimum* (1%, w/w, CMS/soil) and sunk into the sand-vermiculite mixture to the depth of the propagation mats. The mixture was then packed around each pot to create a temperature-stable environment. All pots were seeded with the parsley cultivar Plain Italian (0.31 g of seed per 10-cm row), watered thoroughly, and maintained at field capacity for the rest of the experiment. When necessary, pots and sand beds were moistened with water of the approximate temperature at which individual beds were maintained.

Treatments were replicated six times using a randomized complete block design. Pots filled with soil mixed with sterile CMS served as controls. Data were collected 14 days after seedling emergence, and the soil from each treatment was air-dried on a greenhouse bench for use in determining pathogen populations. Once air-dried, the population levels of *Pythium* spp. were determined with the plate-dilution frequency/gallic acid medium method described by Lumsden et al (13).

The relative saprophytic activity of *R. solani* was estimated using a modification of a procedure described by Papavizas et al (17). The modification involved the use of the selective medium developed by Ko and Hora (7) as opposed to antibiotic-fortified WA.

In environmental chamber tests, 10-cm paper pots were filled with uninfested soil or soil infested with oat cultures (1:1, w/v, oats/water) of either *R. solani* AG-4 (1%, w/w, oats/pasteurized soil) or *P. irregulare-P. ultimum* (0.5%, w/w, oats/soil). Pots with soil were then placed in one of three environmental chambers set at 20/10 ± 1, 25/15 ± 1, or 30/20 ± 1 C (day/night temperatures). Environmental chambers had 12-hr photoperiods of both fluorescent and incandescent light alternated with 12 hr of darkness. Pots were seeded, maintained, and evaluated in the manner described for sand bed studies. All treatments were replicated six times using a randomized complete block design.

RESULTS

Field survey. On the basis of field observations, the incidence and severity of postemergence damping-off increased from May to a peak during August. Postemergence damping-off usually killed small or large portions of seedling rows within beds. In instances where damping-off was mild, seedling rows were thinned but not completely killed. In either case, stand densities in certain fields of both plain- and curl-leaved parsley were often reduced to levels well below those acceptable to growers. There were no substantial differences between the levels of disease observed for either plain- or curl-leaved parsley.

Seedlings with symptoms of postemergence damping-off were characterized by the presence of brown-gray to orange water-soaked lesions of both primary and secondary roots. Epicotyl collapse generally followed lesion development and appeared to be in response to root tissue disorganization.

Isolation of fungi. Throughout this study, 1,420 fungal cultures were isolated from field-grown parsley seedlings showing symptoms of postemergence damping-off. The most commonly isolated fungi (Table 1) were *P. ultimum, P. irregulare, F. oxysporum* Schlecht., *F. solani* (Mart.) Appel & Wollenw., and *R. solani*. The remaining isolates were distributed among six additional taxa. There were only slight differences between the frequencies of fungi isolated from curl- and plain-leaved cultivars during these studies (Table 1).

More than 70% of a random sample of 127 *Pythium* isolates were identified as *P. ultimum*, more than 20% were *P. irregulare*, and the remaining isolates were identified as *P. spinosum* Savv., *P. paroecandrum* Drechs., and *P. debaryanum* Hesse. Several isolates could not be identified because of our inability to induce them to form sexual structures.

Of 32 isolates of *R. solani* assigned to anastomosis groupings, 18 (56%) were of the Praticola-type (AG-4), seven (22%) were of the Rush-type (AG-2, type 2), and seven (22%) were able to anastomose with AG-2 testers. No isolates belonged to the Crucifer-type (AG-2, type 1) or to AG-3. Although isolates within groups were fairly consistent with respect to their cultural characteristics on PDYCA, they did show considerable variation in coloration, aerial mycelium formation, and sclerotial characteristics and abundance.

Pathogenicity of isolates. As a group, isolates of *Pythium* spp. were the most pathogenic to parsley seedlings and resulted in the highest pathogenicity ratings and the lowest dry weight determinations, respectively (Table 2). Most isolates of *P. irregulare* and *P. ultimum* caused severe preemergence damping-off under greenhouse conditions. Postemergence damping-off, however, was generally severe on seedlings that were not killed before emergence. No other species of *Pythium* tested caused disease under the conditions of experiments. Both plain- and curl-leaved cultivars reacted similarly when inoculated with pathogenic isolates of *Pythium* spp.

The symptomatology of postemergence damping-off caused by pathogenic isolates of *Pythium* spp. ranged from the formation of dark brown to orange-brown lesions on both primary and secondary roots. Hypocotyl discoloration of an orange hue was occasionally observed. Generally, epicotyl tissue became flaccid and collapsed subsequent to root disorganization. At this stage, fungus mycelium was usually abundant throughout the intercellular and intracellular matrices of infected tissues.

Isolates of *R. solani* were the next most pathogenic to parsley seedlings (Table 2). AG-2, type 2, isolates and, to a lesser extent, AG-4 isolates were the most virulent of the *R. solani* isolates tested. All of the AG-2, type 2, isolates caused severe damping-off (mostly postemergence), whereas AG-4 isolates ranged from nonpathogenic to highly pathogenic. AG-1 isolates were mostly avirulent or slightly virulent; however, three isolates tested did incite severe postemergence damping-off. As with *Pythium* spp., the pathogenicity of *R. solani* isolates did not differ on the two cultivars tested.

The symptomatology of postemergence damping-off caused by pathogenic isolates of *R. solani* was similar to that described for *Pythium*. Stem lesions, however, were generally dark to light brown and usually occurred at or near the soil line.

Nearly all isolates of *F. solani* and *F. oxysporum* were avirulent on both

Table 1. Fungi isolated from lesions in field-grown parsley seedlings with symptoms of postemergence damping-off

Fungus	Frequency of isolation (%)	
	Curl-leaved	Plain-leaved
Fusarium oxysporum	24	19
F. solani	12	15
Pythium spp.[a]	30	34
Rhizoctonia solani[b]	11	12
Other fungi[c]	23	19
Total number of isolates	548	872

[a] *P. ultimum* = 72.3%, *P. irregulare* = 22.1%, *P. debaryanum, P. paroecandrum, P. spinosum,* and *Pythium* spp. = 6.6%.
[b] Anastomosis group (AG) 4 = 56%, AG-2, type 2 = 22%, AG-1 = 22%, and AG-2, type 1, and AG-3 = 0%.
[c] Other fungi included *Acremonium* spp., *Alternaria* spp., *Aureobasidium* spp., *F. moniliforme, F. roseum, Phoma* spp., *Sclerotinia* spp., and *Stemphylium* spp.

Table 2. Pathogenicity classes of fungi isolated most frequently from damped-off parsley seedlings in New Jersey

Fungus[a]	Number tested	Number of isolates in pathogenicity classes[b]							Class mean	Dry weight (% of control ± SD)	
		1	2	3	4	5	6	7		Curl-leaved	Plain-leaved
Fusarium oxysporum	23	17	6	–	–	–	–	–	1.26	97.8 ± 4.4	97.3 ± 2.3
F. solani	21	16	5	–	–	–	–	–	1.24	97.9 ± 2.1	96.3 ± 1.0
Pythium irregulare	19	–	–	–	–	2	8	9	6.37	43.0 ± 5.0	38.8 ± 6.1
P. ultimum	17	–	–	–	3	1	6	7	6.00	44.1 ± 3.3	39.2 ± 5.1
Rhizoctonia solani AG-1	8	3	2	–	–	1	1	1	3.13	67.7 ± 5.4	72.1 ± 7.0
R. solani AG-2, type 2	7	–	–	–	–	–	1	6	6.86	40.0 ± 1.7	37.3 ± 6.9
R. solani AG-4	18	1	–	–	2	4	4	7	5.94	47.7 ± 2.2	49.0 ± 4.3
Other fungi[c]	59	55	3	1	–	–	–	–	1.08	98.1 ± 0.7	99.7 ± 5.5
Total number tested	172										

[a] Cornmeal-sand (CMS) cultures were incorporated into soil at a rate of 1:100, w/w, CMS/soil.
[b] 1 = No disease, 2 = one-third of plants discolored, 3 = two-thirds discolored, 4 = more than two-thirds discolored, 5 = one-third damped off, 6 = two-thirds damped-off, 7 = more than two-thirds damped-off.
[c] Other fungi included *Acremonium* spp., *Alternaria* spp., *Aureobasidium* spp., *F. moniliforme, F. roseum, P. aphanidermatum, P. debaryanum, P. paroecandrum, P. spinosum, Sclerotinia* spp., and *Stemphylium* spp.

parsley cultivars (Table 2); however, several isolates of both species were associated with slight hypocotyl and root discoloration in some plots. These isolates were rarely reisolated from diseased tissue. Similar results were obtained for species of *Stemphylium* and *Alternaria*. Isolates of 14 other genera and/or species of fungi were nonpathogenic to parsley seedlings.

Temperature-pathogencity studies. In temperature-controlled sand bed studies (Fig. 1), *R. solani* was less pathogenic at 15 C than at either 23 or 30 C. *P. irregulare-P. ultimum* was highly pathogenic at 15, 23, and 30 C.

Generally, parsley germinated faster at 23 and 30 C than it did at 15 C (Fig. 2). Aside from influencing the rate of seedling emergence, temperature had no effect on either percent emergence or growth characteristics of developing seedlings after emergence.

When the saprophytic activity of *R. solani* was determined for the various treatments (Fig. 1), the percentage of table beet seed colonized was 85% at 15 C and 100% at both 23 and 30 C. The inoculum density of *P. irregulare-P. ultimum* after the sand bed test was 585 parts per gram (ppg) of air-dried soil at 15 C, 698 ppg at 23 C, and 489 ppg at 30 C.

In environmental chamber studies (Fig. 3), *R. solani* was moderately pathogenic in the range of 10–20 C, with the number of surviving plants (as a percentage of uninfested controls) substantially higher than that of other plots. *P. irregulare-P. ultimum* was highly pathogenic compared with *R. solani* within the same temperature range. Conversely, at 15–25 and 20–30 C, both *P. irregulare-P. ultimum* and *R. solani* were highly pathogenic compared with uninfested controls. The number of surviving plants was similar for the two treatments.

DISCUSSION

Under field conditions, damping-off of parsley frequently reduced stand densities of both plain- and curl-leaved parsley to well below acceptable levels. This presents a major problem to growers, because stand density is the primary factor affecting yields and profits.

According to the frequency of isolation from diseased seedlings and the results of pathogenicity tests, the primary cause of parsley damping-off in southern New Jersey is *P. ultimum* and, to a lesser extent, *P. irregulare*. These fungi have not previously been reported as pathogens of parsley. Both species were highly pathogenic on parsley and caused primarily preemergence damping-off under greenhouse conditions. This agrees with reports for other crops (10,12) where preemergence damping-off was the predominant form of damping-off caused by these fungi in greenhouse tests. In our studies, *P. ultimum* and *P. irregulare* were highly pathogenic at temperatures ranging from 15 to 30 C in both temperature-controlled sand beds and environmental chambers (Figs. 1 and 3). This corresponds with temperature ranges necessary for disease development by *P. ultimum* in other hosts (6,8). However, there is a great deal of variation in the literature regarding the influence of temperature on disease caused by these fungi (8,18,21). This variability may result from differing experimental conditions as well as the complex relationship between temperature, moisture, pathogen, and host (9).

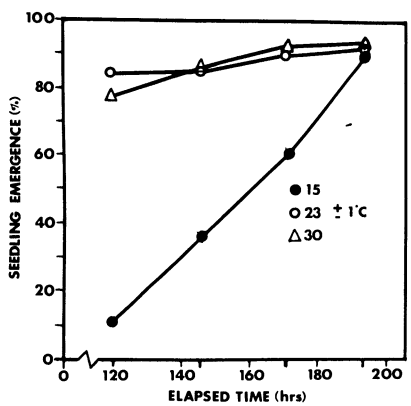

Fig. 2. Influence of three temperatures on seedling emergence of parsley in temperature-controlled sand beds over time. Data are the means of six replicates.

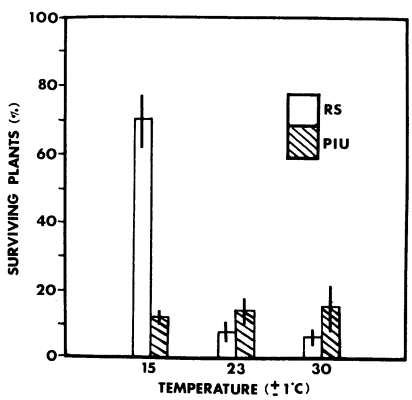

Fig. 1. Influence of three temperatures on damping-off of parsley in temperature-controlled sand beds. RS = *Rhizoctonia solani* AG-4 cornmeal-sand inoculum (CMS) incorporated into pasteurized field soil (1%, w/w, CMS/soil). PIU = *Pythium irregulare-P. ultimum* CMS inoculum added to soil (1.5%, w/w, CMS/soil). Data are the means of six replicates. Bars indicate one standard deviation.

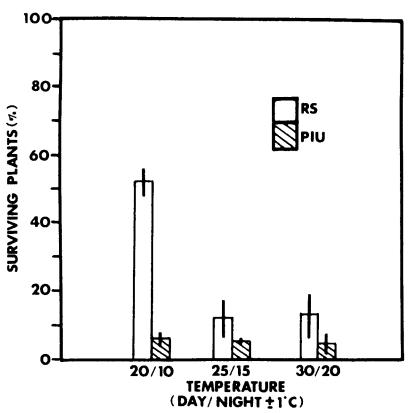

Fig. 3. Influence of three temperature regimes on damping-off of parsley in environmental chambers. RS = *Rhizoctonia solani* oats inoculum incorporated into pasteurized field soil (1.9%, w/w, oats/soil). PIU = *Pythium irregulare-P. ultimum* oats inoculum incorporated into soil (1%, w/w, oats/soil). Data are the means of six replicates. Bars indicate one standard deviation.

P. debaryanum, though reported to have been the cause of moderate damping-off of parsley in Michigan (2), was rarely isolated in New Jersey. Moreover, an isolate of *P. debaryanum* did not incite damping-off when tested for pathogenicity.

In addition to *P. ultimum* and *P. irregulare, R. solani* is considered to be an important cause of parsley damping-off. Isolates of AG-1 were generally less pathogenic and had a greater range of pathogenicity than isolates of either AG-4 or AG-2, type 2 (Table 2). Dodman et al (3) reported that most isolates of AG-1 tend toward a mechanism of host penetration that is characterized by the production of lobate appressoria and that isolates of AG-2 and AG-4 tend toward a mechanism that involves the formation of dome-shaped infection cushions. It may be that the differences in pathogenicity between the various anastomosis grouping types are at least in part related to their varying modes of host penetration.

R. solani AG-4 was highly pathogenic on parsley only at temperatures ranging from 23 to 30 C (Figs. 1 and 3). This is consistent with reports for this pathogen on other hosts (5,19) where disease is less severe at low temperatures.

It has been suggested that the main influence of temperature on certain soilborne diseases may be related more to its effect on the growth and physiology of the host than that of the pathogen (9). This is supported by the results of our *R. solani* sand bed studies (Fig. 2), where low temperature was found to depress the rate of seedling emergence and disease severity even though the saprophytic activity of *R. solani* was nearly as high as that in heavily diseased plots. Low temperatures may have conferred a certain degree of resistance or tolerance to parsley that resulted in reduced levels of disease.

Our findings indicate that parsley damping-off in southern New Jersey is attributed to *P. ultimum, P. irregulare,* and *R. solani* AG-2, type 2, and AG-4. *Fusarium* spp. were reported by Walker (23) to cause significant root rot and damping-off of parsley in southern New Jersey, but none of our *Fusarium* isolates were pathogenic to parsley. We found that damping-off can occur over the temperature range of 15–30 C with *Pythium* spp. being the predominant pathogens at the lower temperatures and both *Pythium* spp. and *R. solani* being active at 23–30 C. These results generally agree with field observations, although disease in the field was typically most severe under the influence of moderate soil temperature (about 25 C) and high soil moisture. This may reflect the adverse effects of these conditions on the activity of soil microflora antagonistic to *Pythium* spp. and *R. solani* (11).

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